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Research Article

Pollination Requirements and the Foraging Behavior of Potential Pollinators of Cultivated Brazil Nut (*Bertholletia excelsa* Bonpl.) Trees in Central Amazon Rainforest

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This study was carried out with cultivated Brazil nut trees (*Bertholletia excelsa* Bonpl., Lecythidaceae) in the Central Amazon rainforest, Brazil, aiming to learn about its pollination requirements, to know the floral visitors of Brazil nut flowers, to investigate their foraging behavior and to determine the main floral visitors of this plant species in commercial plantations. Results showed that *B. excelsa* is predominantly allogamous, but capable of setting fruits by geitonogamy. Nineteen bee species, belonging to two families, visited and collected nectar and/or pollen throughout the day, although the number of bees decreases steeply after 1000 HR. Only 16, out of the 19 bee species observed, succeeded entering the flower and potentially acted as pollinators. However, due to the abundance, flower frequency and foraging behavior of floral visitors, it was concluded that only the species *Eulaema mocsaryi* and *Xylocopa frontalis* could be considered relevant potential pollinators.

1. Introduction

Brazil nut (*Bertholletia excelsa* Bonpl., Lecythidaceae) is native from the Amazon forest occurring in the wild from 5°N to 14°S in Venezuela, Colombia, Peru, Bolivia, Suriname, Guyana, and Brazil [1–3]. It is harvested for its nut, which is extracted from inside the large, rounded and hard-to-break fruit collected on the ground after falling from the trees [4]. Most production is for export comprising an important source of food and income to the indigenous people [5].

Brazil nut is believed to be an allogamous species presenting mellitophilous pollination syndrome, thus depending on biotic pollinators to set fruits [6]. However, little is known about its breeding system and pollination requirements. The blooming period occurs from September to December, peaking in November, and flowers are produced profusely in vertical terminal panicles [6, 7]. The flower is large (c.a. 3.9 cm in length × 3.6 cm in width), zygomorphic, with two to three sepals, and six yellowish petals [6, 8]. It bears a curled hood made of congruent staminodes, called ligule, that in association with the petals form a chamber which conceals stamens, stigma, and nectaries [8, 9]. The large size and strength of the hood restricts and selects flower visitors to medium- and large-sized bees strong enough to uncurl it [7, 8]. Anthers begin to dehisce while the flower is still closed, around 0100 HR-0130 HR and over 90% of anthers are shedding pollen by 0300 HR. Pollen viability ranges from 76% to 86.5% and remained viable until 1400 HR [10, 11]. Anthesis takes place between 0430 HR to 0500 HR, and petals fall off after 24 h. When fecundation does not occur, the pistil drops after 48 h [10]. The ovary bears an average of 20 ovules, and only 0.28 to 0.40% of the flowers produced set

fruits [12, 13]. Fruits take an average of 15 months to mature [7, 14].

There are few studies investigating floral visitors of Brazil nut, and usually they are restricted to the genus level. Prance and Mori [15] stated that the main pollinators of species belonging to the Lecythidaceae family are *Bombus* and *Euglossa* bees. Müller et al. [10], dealing with *B. excelsa*, believe that large-sized bees of the genus *Bombus* are the main pollinators of this species, while a study carried out in Bolivia, suggested that euglossine bees are the effective pollinators [13]. However, a study carried out in the state of Acre, Brazil, points out to bees of the genus *Xylocopa* [16]. Only Nelson et al. [9] in a study nearby the city of Manaus, State of Amazonas, and Maués [7], working close to the city of Belém, State of Pará, have identified the bee species visiting Brazil nut flowers to the species level. In both cases, they were all medium-to large-sized bees: *Eulaema seabrai* (Moure, 1960), *Epicharis rustica* (Olivier, 1789), *Ep. umbraculata* (Fabricius, 1804), *Eulaema nigrita* (Lepeletier, 1841), *El. cingulata* (Fabricius, 1804), in Nelson et al. [9] work, and *Xylocopa frontalis* (Olivier, 1789), *X. aurulenta* (Fabricius, 1804), *Ep. rustica* (Olivier, 1789), *Ep. affinis* (Smith, 1874), *Centris similis* (Fabricius, 1804), *El.nigrita, El. cingulata, Bombus brevivillus* (Franklin, 1913), and *B. transversalis* (Olivier, 1789), in Maués [7] report. Recently, Santos and Absy [17] reported *X. frontalis* and *El. mocsaryi* (Friese, 1899), as the most abundant floral visitors of *B. excelsa* flowers in Itacoatiara county, State of Amazonas.

There is a lack of precise information on the breeding system and floral visitors of *B. excelsa*. This work aimed to investigate the pollination requirements, learn about the identity and foraging behavior of visitors to Brazil nut flowers, and discuss their potential as pollinator of this plant species. Such knowledge is remarkably important in developing policies of sustainable use of the forest and conservation of the native bee pollinators. It may also help to explain and to overcome the low productivity observed in commercial plantations of Brazil nut [8–10].

2. Methods

The experiment was carried out in Aruanã farm, situated on the road Manaus-Itacoatiara, km 215, county of Itacoatiara, State of Amazonas, Brazil, at 3° 0′ 30.63″ S and 58° 50′ 1.50″ W. The farm total area comprises 12,000 ha, of which 3,600 are cultivated with 20 varieties of grafted Brazil nut trees. The trees are spaced at 20 × 20 m reaching approximately 1,300,000 trees. It is the largest Brazil nut plantation in the world.

Four trees (three belonging to variety 609 and one to variety Abufari) were chosen at random out of those in blooming. These trees were ca. 0700 HR apart from each other and ranged from 25–30 m in height. Scaffolds were built by the side of each tree, allowing to spot visually 60% of their canopies and access flowers for data collection. Field observations were carried out for 78 days, from October to December 2007, covering the whole flowering period, especially its peak in November.

2.1. Pollination Requirements. Aiming to know the pollination requirements of Brazil nut trees and the role of bees in pollinating this plant species, we applied five pollination treatments to the trees during their blooming.

T1: Open Pollination. We marked 655 buds with satin threads tied to their petiole in the day before flower anthesis. These buds were observed throughout the anthesis and flower lifespan until they have fallen from the trees or being set, until 25 days later. In this treatment, we aimed to know the natural levels of pollination of Brazil nut trees in the area studied.

T2: Restricted Pollination. 326 buds were covered with muslin bags and remained bagged for 25 days. The aim of this treatment was to verify the dependence or nondependence of Brazil nut flowers on biotic pollination.

T3: Hand Cross-Pollination. 150 buds were marked with satin threads and bagged with muslin bags. Next day, after anthesis, flowers were unbagged and manually pollinated with pollen grains from flowers of another Brazil nut tree being deposited directly on the stigma. Donor flowers were collected minutes before we start to perform hand pollination and taken immediately to receptor tree. Then, pollen grains were removed from the anthers of the donor flower using a fine painting brush and transferred promptly to the stigma of the receptor flower. Immediately after hand-pollinated, the flowers were protected with muslin bags for 25 days. This treatment indicates cross-pollination requirements of the brazil nut tree and the existence any pollination deficit by comparison to natural fruit set in the area (open pollination).

T4: Hand Self-Pollination. We marked 98 buds and followed the same procedure described above, except that pollen grains were transferred between anthers and stigma of the same flowers. In this treatment, results show if the Brazil nut tree is self-compatible or not.

T5: Geitonogamy. The same procedure above was repeated here with 78 buds, but pollen grains were transferred from anthers of a flower to the stigma of a different flower from the same tree. We aimed to learn if the Brazil nut tree shows any sort of incompatibility, this kind of crossing and, its dependence on foreign pollen grains.

In this experiment, colors of the satin threads varied according to the treatment, and satin threads were carefully tied to the buds' petiole avoiding damaging the buds, obstruction of the anthesis, and normal development of the flower and fruit set. Also, all hand pollinations were performed between 0600 HR and 0800 HR when, according to Müller et al. [10], fecundity is greatest.

Brazil nut fruits take an average of 14 months to ripe, and other factors besides pollination can interfere with fruit persistence on trees [7, 14]. Thus, in all tests we assessed initial fruit set 25 days after flower manipulation as a measure of pollination effectiveness. This is a reliable measure because unpollinated flowers fall from the trees in the same day they

open, while pollinated ones remain on the trees and show an ovary about 1.5 mm in diameter 25 days later.

2.2. Floral Visitors and Foraging Behavior. Samples of all floral visitors were collected from each tree using entomological nets at every hour from 0500 HR to 1700 HR. Then, insects were killed in a lethal chamber with ethyl acetate, pinned, identified at species level and, sexed, and counted to determine their specific abundance.

During blooming, the foraging behavior of each flower visiting species was recorded considering the following parameters: frequency, abundance, hour of the day and number of visits, time spent per flower, approach and handling of the flower, and entry to the flower. Data were collected using a notepad, a stop watch, a video and photo camera Sony Cyber-shot DSC-H50 9.1 MP, and by means of visual observation of the bees foraging on the flowers, most of them are out of the reach of the observer but in his sight. Recording was initiated when the bee species arrived to the tree and stopped when the insect flew away or went out of the observer's sight, that was limited to only part of the canopy. All data were collected in 25 periods of 30 minutes each, starting at 0500 HR and ending by 1700 HR. This information was later related to temperature, and air relative humidity records obtained every 30 minutes using a digital thermal hygrometer, model Impac TH02, because there are evidences that increases in ambient temperature have a negative impact o the foraging of bees [18, 19].

2.3. Statistical Approach. Data on pollination requirements did not conform to the ANOVA presumptions due to their binomial character (set fruit or nonset fruit) and were analysed using the nonparametric test of Kruskall-Wallis, and means were compared by the nonparametric Dunn's test.

Data regarding the number of flowers visited per tree and time spent per flower were analysed by ANOVA, and means were compared *a posteriori* by Tukey test at 5%. All tests were performed using SPSS 19 Statistics program.

3. Results

3.1. Pollination Requirements. There were significant ($P < 0.05$) differences between treatments for fruit set (Table 1). The hand cross-pollination treatment set the greater number of fruits and differed ($P < 0.001$; KW = 54.295) from all other treatments, while the geitonogamy treatment did not differ ($P < 0.001$, KW = 54.295) to the free pollination treatment. Flowers submitted to the restricted and hand self-pollination treatments set no fruits (Table 1).

3.2. Flower Visitors and Foraging Behavior. Flowers of *B. excelsa* were visited by a wide range of animals, such as Hymenoptera (bees), Lepidoptera (butterflies and moths), and birds (hummingbirds). In Hymenoptera, a great variety of bee species was observed and collected visiting Brazil nut flowers. These bees belonged to two families (Apidae and Megachilidae) in a total of 19 species (Table 2).

Observations on the foraging behavior of floral visitors and potential pollinators showed that bees collect both pollen and nectar from *B. excelsa* flowers. The place from where bees collected nectar from the flowers varied according to the species size. Larger bee species harvested nectar from the ligule base, while smaller species got inside the flower to collect the nectar present at the base of the anthers.

Table 1: Initial fruit set of Brazil nut (*Bertholletia excelsa*) flowers submitted to five pollination treatments: open pollination, bagged with muslin bags, hand cross-pollination, hand self-pollination, and geitonogamy. Itacoatiara, Amazonas, Brazil, 2007.

Treatments	n	Fruit set (number)	Fruit set (%)
Free pollination	655	20	3.05[b]
Pollinator exclusion	326	0	0
Hand cross-pollination	159	29	19.33[a]
Hand self-pollination	98	0	0
Geitonogamy	78	3	3.85[b]

[*] Values followed by the same letters are not significantly different ($P < 0.001$; Kruskal-Wallis ANOVA).

Bees initiated harvesting pollen and nectar at 0515 HR and reached a peak of foraging activity between 0530 HR and 0600 HR. After 1000 HR the number of bees foraging on flowers dropped steeply, coinciding to the temperature increase and relative air humidity drop (Figure 1). However, a small number of bees kept foraging in the afternoon, specially the species *Xylocopa frontalis*. On the contrary of Müller et al. [10] report of bees starting to forage earlier in the dawns following full moon nights, we did not register any difference from the other nights ($n = 2$).

The most abundant floral visitor of Brazil nut was the carpenter bee *Xylocopa frontalis*. This species was the first one to arrive at the flowers (around 0515 HR) to collect nectar and pollen (Figure 2(a)) and was found in great numbers and frequency throughout the whole blooming season of the trees studied. After reaching a flower, *X. frontalis* was used to make a brief inspection of it and, if not rejected, pushed inside the flower using its ligule as a platform to collect nectar from the base of the ligule itself. This bee species was, apparently, the one which carried more pollen on its body, especially on the back of the thorax, head, and in the scopa. A typical behavior observed in *X. frontalis* while foraging was to sit on a flower and groom pollen out of its body towards the scopa and discard with the forelegs the exceeding pollen grains. *Xylocopa frontalis* was among the three bee species that visited most flowers per tree and spent over than 10 seconds per visit (Table 3). Males were observed visiting flowers for nectar, but they also carried great amounts of pollen on their thorax (Figure 2(b)).

Centris denudans (Lepeletier, 1841) was observed visiting flowers (Figure 2(h)) in the canopy of all trees of this study. It was present throughout the blooming season, carrying small amounts of pollen on the back of the thorax, despite the bee large size. This species frequently chased after other individuals of the same species in quick flights over the canopy, possibly to drive the other bee off the food source or to mate with her. It was one of the few species observed foraging in the afternoon, the hottest part of the day,

Table 2: List of families, species, sex and body size of bees, floral visitors, and potential pollinators of Brazil nut (*Bertholletia excelsa*), collected in a commercial cultivation in the county of Itacoatiara, state of Amazonas, Brazil, 2007.

Family	Species	Sex	Body size (mm) ± s.d.
Apidae	*Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier, 1789)	♂♀	34.60 ± 0.10
Apidae	*Epicharis* (*Epicharana*) *flava* (Friese, 1900)	♀	17.40 ± 0.26
Apidae	*Epicharis* (*Epicharana*) *conica* (Smith, 1874)	♂♀	12.30 ± 0.97
Apidae	*Epicharis* (*Epicharis*) *umbraculata* (Fabricius, 1804)	♀	28.70 ± 1.10
Apidae	*Epicharis* (*Parepicharis*) *zonata* (Smith, 1854)	♀	15.20 ± 0.75
Apidae	*Centris* (*Ptilotopus*) *americana* (Klug, 1810)	♀	35.10 ± 0.88
Apidae	*Centris* (*Trachina*) *carrikeri* (Cockerell, 1919)	♂	5.50 ± 1.04
Apidae	*Centris* (*Xanthemisia*) *ferruginea* (Lepeletier, 1841)	♀	7.80 ± 0.45
Apidae	*Centris* (*Ptilotopus*) *denudans* (Lepeletier, 1841)	♂♀	34.20 ± 1.75
Apidae	*Eulaema* (*Eulaema*) *meriana* (Olivier, 1789)	♂♀	33.40 ± 1.20
Apidae	*Eulaema* (*Apeulaema*) *mocsaryi* (Friese, 1899)	♂♀	15.60 ± 0.84
Apidae	*Eulaema* (*Apeulaema*) *cingulata* (Fabricius, 1804)	♀	14.60 ± 0.93
Apidae	*Bombus* (*Fervidobombus*) *transversalis* (Olivier, 1789)	♀	16.40 ± 2.86
Apidae	*Eufrisea purpurata* (Mocsáry, 1896)	♀	10.80 ± 0.89
Apidae	*Eufrisea flaviventris* (Friese, 1899)	♀	15.30 ± 1.33
Apidae	*Apis mellifera scutellata* (Lepeletier, 1836)	♀	4.40 ± 0.19
Apidae	*Frieseomelitta longipes* (Smith, 1854)	♀	1.50 ± 0.24
Apidae	*Melipona* (*Michmelia*) *lateralis* (Erichson, 1848)	♀	4.90 ± 0.32
Megachilidae	*Megachile* sp. 1	♀	4.65 ± 0.76

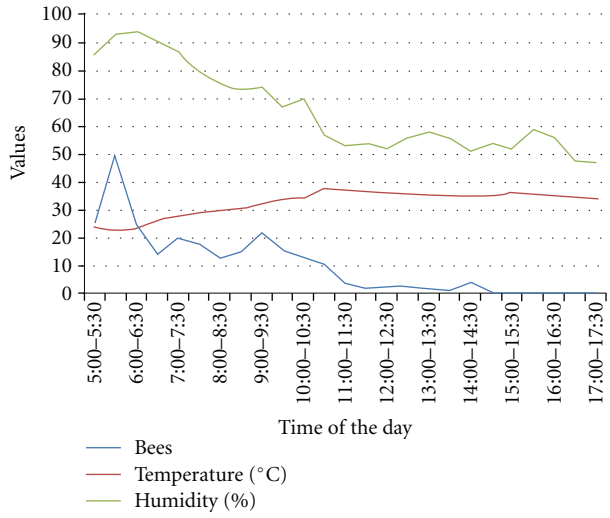

Figure 1: Frequency of floral visitors associated to temperature and relative humidity (at each 30 minutes) in a commercial cultivation of Brazil nut (*Bertholletia excelsa*) in the county of Itacoatiara, state of Amazonas, Brazil, 2007.

although most of its foraging activities were recorded in the morning. This bee species approached the flowers in a different way of *X. frontalis* because it did not inspect and rarely rejected a flower, entering the flower immediately after reaching it, but also harvested nectar from the ligule base. *Centris denudans* ranked second among the species that visited most flowers per tree, usually flowers close to each other, and also spent over than 10 seconds per flower visit (Table 3). Males were observed and recorded visiting *B. excelsa* flowers, and mating events on Brazil nut flowers were also registered.

Eulaema meriana (Olivier, 1789) was also present throughout the blooming season, but only in the morning. Like *X. frontalis*, frequently rejected some flowers but always carried large amounts of pollen in its corbicula. Due to its large glossa, this bee species also collected nectar from the ligule base landing on the ligule itself (Figure 2(k)). *El. meriana* was the bee species that visited most flowers per tree, usually neighboring flowers, spending over than 16 seconds per visit (Table 3). Males of this species were observed harvesting nectar from the Brazil nut flowers.

Centris americana (Klug, 1810) was seen only in some moments of the blooming period and always in small numbers and low frequency to flowers, never exceeding one individual per tree at a given time. This species approached the flower like the other large-sized bees, using the ligule as a platform for landing and collecting nectar from the ligule base (Figure 2(j)). It spent less than 8 seconds per visit (Table 3).

Bombus transversalis was recorded only in the beginning of the blooming season (Figure 2(o)). It was one of the species that spent most time per flower visit, reaching up to 90 seconds inside a flower in some visits. Despite staying long in the flower, *B. transversalis* usually transported small

Figure 2: Approach to flowers of Brazil nut (*Bertholletia excelsa*) by distinct bee species in a commercial cultivation in the county of Itacoatiara, state of Amazonas, Brazil, 2007. ((a); (b)) *Xylocopa frontalis* (♀ and ♂, resp.); (c) *Epicharis (Epicharana) flava* (♀); ((d); (e)) *Epicharis (Epicharana) conica* (♀ and ♂, resp.); (f) *Epicharis (Epicharis) umbraculata* (♀); (g) *Epicharis (Parepicharis) zonata* (♀); (h) *Centris (Ptilotopus) denudans* (♀); (i) *Centris ferruginea* (♀); (j) *Centris (Ptilotopus) americana* (♀); (k) *Eulaema (Eulaema) meriana* (♀); ((l); (m)) *Eulaema* (*Apeulaema*) *mocsaryi* (♂ and ♀, resp.); (n) *Eulaema* (*Apeulaema*) *cingulata* (♀); (o) *Bombus* (*Fervidobombus*) *transversalis* (♀); (p) *Eufriesea flaviventris* (♀); (q) *Megachile* sp.1; (r) *Frieseomelitta longipes* robbing pollen from *El.* (A.) *mocsaryi*.

amounts of pollen and visited only a few flowers per tree (Table 3). Due to its medium size, this species entered almost entirely in the flower to collect nectar at the ligule base.

Eulaema mocsaryi was the second most abundant and frequent species over the whole blooming season, mainly in the morning shift (Figure 2(m)) but also observed visiting flowers in the afternoon. It frequently rejected flowers that possibly had been previously visited by other bee. Between two flower visits, while in flight or landing on a leaf, individuals of this species combed pollen from their bodies into the corbicula making large pollen loads. This bee visited less than five flowers per tree moving quickly to other trees

Table 3: Bee relative abundance, mean number (± standard error: SE) of flowers visited per tree by ten bee species and mean time (± standard error: SE), in seconds, spent by twelve bee species per visit to flowers of Brazil nut (*Bertholletia excelsa*) variety 609, under cultivation in the Amazon rainforest (*n*: number of bees recorded per species).

Species	Relative abundance	Number of flower visits per tree			Time spent per flower visit		
	(%)	*n*	X ± S.E.		*n*	X ± S.E.	
Xylocopa frontalis	62.85	136	11.33 ± 0.834	abc	64	11.63 ± 0.754	bcd
Centris denudans	6.84	35	14.71 ± 2.368	bc	64	11.96 ± 0.736	bcd
Centris americana	1.11		—		4	7.73 ± 0.694	cd
Centris ferruginea	0.55	3	3.67 ± 2.667	c	31	9.14 ± 0.854	cd
Eulaema meriana	6.65	17	15.10 ± 2.358	a	57	16.05 ± 1.204	bc
Eulaema mocsaryi	12.20	72	4.36 ± 0.514	abc	48	15.34 ± 1.488	bc
Eulaema mocsaryi (male)		9	8.33 ± 2.677	abc	55	5.68 ± 0.265	d
Epicharis conica	3.88	8	2.75 ± 0.773	c	7	18.39 ± 2.714	bcd
Epicharis flava	0.37	7	4.43 ± 1.288	bc	45	11.86 ± 1.354	bcd
Epicharis zonata	0.92	9	1.67 ± 0.289	c	3	31.38 ± 13.090	a
Eufrisea flaviventris	0.37	6	7.33 ± 3.373	abc	58	5.96 ± 0.983	d
Eufrisea purpurata	0.74		—		4	14.54 ± 5.809	bcd
Bombus tranversalis	3.51	3	6.33 ± 2.963	abc	42	27.61 ± 1.928	a

* Values followed by the same letters are not significantly different ($P < 0.005$; ANOVA).

(Table 3). However, when visiting a flower, *El. mocsaryi* spent over 15 seconds increasing the chance to deposit pollen on the stigma (Table 3).

Epicharis conica (Smith, 1874) was present throughout the blooming season and like *El. mocsaryi* was more frequent in the morning shift, but also present in the afternoon. Due to its small size, this species penetrates the flower almost entirely and unlike the previous species present here, the bee makes a turn inside the flower before leaving it facing out (Figure 2(d)). This bee was the second species that visited less flowers per plant, but took over 18 seconds per visit ($n = 7$) (Table 3). Males also visited flowers and pushed their bodies completely through the petals getting hidden by the ligule while inside the flower (Figure 2(e)). Because of this behavior, their presence was only noticed because the buzzing noisy produced when approaching the flower.

Epicharis flava (Friese, 1900) was present in reduced numbers and only when most trees were in bloom. It carried much pollen on the back of the thorax (Figure 2(c)), outstanding as a potential pollinator of Brazil nut flowers. This bee visited few flowers per tree and spent around 12 seconds per visit (Table 3).

Epicharis zonata (Smith, 1854) is a small bee that like other species of its size gets inside the flower becoming hidden from sight and leaves it facing out carrying small amounts of pollen on its body (Figure 2(g)). This bee was only found in the peak of the blooming season, mainly around 0900 HR. It is a fast-flying bee that moves between trees frequently making difficult to track its path over a single tree canopy. As a consequence, *Ep. zonata* produced the smaller number of flowers visited per tree among all bee species observed in this study, compensated for the longest period of time registered for flower visit (Table 3).

Eufriesea flaviventris (Friese, 1899) is a medium-sized, fast-flying species, and the faster flower visitor observed in this study spending around only six seconds per visit (Table 3), but many times revisiting consecutively the same Brazil nut flower. This was the only species observed to collect exclusively pollen (Figure 2(p)). It also rejected flowers previously visited by other bees and combed the pollen from its thorax to the corbicula while in flight.

Centris ferruginea (Lepeletier, 1841) is a fast-flying, small-sized bee that penetrates the flower almost entirely using the ligule as a platform. It also leaves the flower facing out (Figure 2(i)) and carrying small amounts of pollen on the back of the thorax. Usually was only noticed due to the buzzing sound of its flight over the canopy. This bee species also visited few flowers per tree, favoring cross-pollination (Table 3).

Megachile sp. was the smaller species registered visiting Brazil nut flowers in this study. It penetrated entirely the flower pushing its body among the petals and ligule and also left the flower facing out with small amounts of pollen on its ventral scopa (Figure 2(q)). Because of its size, probably collected nectar from the base of the anthers and stigma, although it s not possible to know for sure because the bee remained hidden inside the flower while sipping nectar. Due to its low frequency and high flight speed, only one visit was registered.

Eulaema cingulata, *Epicharis umbraculata*, *Centris carrikeri* (Cockerell, 1919), and *Eufriesea purpurata* (Mocsáry, 1896) were collected and observed visiting Brazil nut flowers; however, only in rare occasions not allowing even photos to be taken for the two latter species.

Melipona lateralis (Erichson, 1848) was seen only once visiting a flower and captured immediately after leaving the

flower. No further sights were possible until the end of the study.

Apis mellifera scutellata (Lepeletier, 1836) was the only nonnative species recorded in this study, constituting an invading bee in the Amazon ecosystem. It was present in small numbers flying over the canopy, mainly early in the morning. Because of its small size and strength, the bee could not pull the ligule back as a platform as did the larger bee species or push herself among the ligule and petals to get inside the flower as done by other medium and small-sized bees and remained flying over the flowers and landing to collect small amounts of pollen fallen on petals or ligule after the visits by larger bees.

Frieseomelitta longipes (Smith, 1854) was found in the trees all over the morning shift and in greater numbers than *A. mellifera* and, for the same reasons, also did not get assessment of the floral resources inside the flower. However, *F. longipes* showed the behavior of trying to rob pollen from the corbicula of large bees in the moment they were visiting the flowers (Figure 2(r)), sometimes making these bees to give up the flower.

Besides bees, butterflies, hawk moths, and hummingbirds were also seen visiting Brazil nut flowers. Butterflies use to land on the flower and insert their long proboscis to collect nectar at any time of the day. Hawk moths were only present early in the morning, around 0430 HR. They hovered in front of the flowers and introduced their proboscis through the petals to collect nectar. Hummingbirds showed no preference for time of the day, visiting flowers at any time and also hovered in front of the flowers to introduce their beak and drink nectar.

4. Discussion

Results showed that *B. excelsa* did not set any fruit in the restricted and hand self-pollination treatments suggesting that this species cannot bear fruits from pollen grains originated from the same flower and requires biotic pollinators to transfer pollen grains between flowers. According to Moritz and Müller et al. [6, 10] the Brazil nut tree does not set from self-fertilization because this mating system led to less than the 85% ovule fertilization necessary for fruit set. However, the geitonogamy treatment produced over 3% of fruit set indicating that the Brazil nut tree can set fruits when pollen grains are transferred between flowers of the same plant. Also, results of the geitonogamy treatment were similar to the open pollination treatment signifying that the pollination achieved in this commercial plantation could be accounted to geitonogamy. These findings, associated to the much greater fruit set following hand cross pollination indicates that the Brazil nut tree is an allogamous species, in accordance to other authors [6, 8–10].

Our results may explain why the individual plant production is much higher in natural clusters of few Brazil nut trees in the forest than in plantations with hundred of trees. In the natural environment, with much fewer flowers to visit, pollinators may be forced to move between trees and revisit flowers in a much more frequent fashion than when they face a seemly unlimited number of blooming trees.

Although many species visit Brazil nut flowers, only some bee species showed foraging behavior compatible to potential pollinators of this tree. While bees were numerous and concentrated their visits to the morning shift, when flowers presented fresh pollen and were more receptive [10], butterflies, and hummingbirds visited inflorescences at any time of the day, in an inconstant pattern and in low numbers. Hawk moths, however, visited flowers in the dawn, close to the sunrise, but were also scarce. Besides that, the great majority of bee species entered and moved inside the flower increasing the chance to transfer pollen from their bodies to the stigmas, while butterflies, hawk moths, and hummingbirds remained outside the flower and introduced a much smaller portion of their bodies, proboscis for the Lepidoptera and beak to the bird, being less likely to deliver pollen to the stigmas. This behavior, in association to the reduced number of individuals, erratic foraging activities, and time of flower visit, suggests that these groups of floral visitors play little or no role in the pollination of *B. excelsa*. On the contrary, the foraging behavior of most bee species indicates that they can be effective pollinators of Brazil nut flowers, in accordance with the suggestions of Prance and Mori [15], Maués and Oliveira [20], Maués [7], Zuidema [13], and Argolo and Wadt [16].

However, some bee species could not enter the flower or did not show a behavior suggestive of relevant pollinators for Brazil nut. The behavior of *Epicharis conica, Ep. zonata, Megachile* sp., and *Centris ferruginea* approaching the flower facing in and leaving it facing out after turning its body inside the flower can contribute to considerable deposition on the stigma of the flower's self-pollen (self-pollination), showed here to produce no fruits. It may happen because the bee leaving the flower facing out can touch the stigma with the back of its thorax, where the pollen has just been placed by the anthers, resulting, at the best, in a mixture of the pollen bees carried from previously visited flowers with that presently visited being deposited on the stigma. In such a situation, these bee species would not be efficient pollinators of Brazil nut flowers because *B. excelsa* is a predominantly allogamous species [6, 7]. Also, *Apis mellifera, Melipona lateralis*, and *Frieseomelitta longipes* did not manage to enter the flowers and could not pollinate them. Besides that, *F. longipes* sometimes prevented flowers to be visited by legitimate pollinators chasing them away for attempting to rob pollen from their corbicula. Although this specific behavior had not been reported before, Santos and Absy [17] showed that the presence of other insects on the flowers can make some floral visitors, presumably pollinators, to avoid these flowers.

Despite potential pollinators, the rare visits of *Eulaema cingulata*, *Epicharis umbraculata*, *Centris carrikeri*, and *Eufriesea purpurata* to Brazil nut flowers suggest that these species contribute little to the pollination of *B. excelsa*. But their presence in the trees may explain why Zuidema [13] pointed out euglossine bees as likely pollinators of *B. excelsa*, although *E. umbraculata*, *C. carrikeri*, and *E. purpurata* had never before been reported as floral visitors of Brazil nut flowers and *E. cingulata* only once in the study by Maués [7].

Although bees of the genus *Bombus* had been suggested as the main pollinators of *B. excelsa* [10, 12], in the present

study only one *Bombus* species, *B. transversalis*, visited the Brazil nut flowers. Nevertheless, these visits were limited to the onset of the blooming season. Therefore, it is likely that the genus *Bombus* does not consist in a relevant taxon for the pollination of *B. excelsa* in the area studied here. Similarly, *Epicharis flava* and *Centris americana* were not abundant and were selective in relation to the blooming stage and probably are not among the main pollinators of Brazil nut flowers.

Bee species like *Centris denudans*, *Eulaema meriana*, *Eufriesea flaviventris*, *Xylocopa frontalis*, and *Eulaema mocsaryi* were frequent in the area during most of the blooming season and showed body size and flower handling adequate to pollinate *B. excelsa* flowers. However, due to the abundance and foraging behavior in the trees, we identified *Eulaema mocsaryi* and *Xylocopa frontalis* as the most relevant pollinators of cultivated *B. excelsa* in Central Amazonia. It is important to stress that, although these two bee species are the ones that potentially most contribute to Brazil nut pollination under the conditions found in this study, the pollination level achieved in the plantation is the sum of the pollination performed by each bee species that constitute that guild of pollinators, including those species that contributed less to the process [21, 22].

Many of the bee species presented in this study as floral visitors and potential pollinators of Brazil nut are widespread in the Amazon region, and some of them also occur in other Brazilian ecosystems [23–25]. Some of these bee species were also reported in the literature interacting with other plant species and constitute important floral visitors or even pollinators. *Eulaema cingulata* is a pollinator to *Ischnosiphon gracilis* (Rudge) Koern (Marantaceae) and floral visitor of *Solanum stramonifolium* Jacq. (Solanaceae) in a fragment of the Atlantic Forest in NE, Brazil [23, 24]. Vilhena and Augusto [25] identified *Ep. flava* as an important floral visitor of *Malpighia emarginata* in a cerrado area of Central Brazil.

In the Amazon, studies carried out in the same area of this work on the floral biology of *Bellucia grossularioides* (Melastomataceae) and floral visitors of *Bixa orellana* (Bixaceae) reported *El. mocsaryi* and *X. frontalis* as the main visitors of these plant species [26, 27]. However, only recently Santos [17] produced the first report suggesting *E. mocsaryi* as an important floral visitor and potential pollinator of *B. excelsa*. Males of *Eulaema meriana* were observed in the present work visiting flowers of Brazil nut to feed on nectar. According to Williams and Whitten [28], these male bees are pollinators of *Catasetum tricomis* (Orchidaceae), suggesting some level of interdependence among these three species because the orchid provides only essences for the male bees of *Eulaema meriana* attract their conspecific females, but the pollen and nectar necessary for the bee survival and reproduction got to be obtained from other plant species, like the Brazil nut.

These observations support the claim of Kremen et al. [29, 30] that conserving the native vegetation on the surrounding of cultivated areas is essential to keep stable populations of pollinators, such as the bees of the present study, for providing food, nesting, and other resources indispensable for their survival. The lack of effective pollinators in numbers adequate to pollinate the large number of flowers present in commercial plantations of Brazil nut can be a cause for the low tree productivity observed in these areas. Among all species identified as potential pollinators of *B. excelsa* in this study only *X. frontalis* have been reared in rational nest boxes and tentatively managed for pollination of passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) in NE Brazil [31]. Investigations on the possibility of rearing and managing *X. frontalis* and other species identified here for pollination of *B. excelsa* are needed.

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